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Production of a Novel OX40 Ligand for Clinical Use

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14. ABSTRACT

Cancer cells have evolved to evade immune-mediated destruction through several documented mechanisms. Our group has developed a technique to enhance immune function in tumor-bearing hosts by targeting a protein on the surface of white blood cells, termed OX40. This type of immune modulation leads to therapeutic benefit in tumor-bearing mice. We have produced a protein that binds to the human OX40 protein and activates human white blood cells. We have a cell line that produces high quantities of this protein and our goal is to test this protein for safety and efficacy in non-human primates so that we can obtain FDA approval for clinical trials in cancer patients. The long-range goal of this proposal is translate these findings to prostate cancer patients.

15. SUBJECT TERMS
T Cells, Co-stimulation, OX40, tumor immunotherapy

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Table of Contents

Page

DOD Prostate Award Research Technical Reporting: 3 year Progress Report
Laboratory-Clinical Transition Award:

PC101977 – Production and Characterization of a Novel OX40 Ligand for Clinical Use.

PI -Andrew Weinberg, PhD

INTRODUCTION: Cancer cells have evolved to evade immune-mediated destruction through several documented mechanisms. Our group has developed a technique to enhance immune function in tumor-bearing hosts through the use of OX40 agonists, which can lead to regression of tumors of various histologies, including prostate cancer. In particular, we have produced a human OX40 agonist, termed OX40L:ILZ:Ig (OX40L:Ig) that has potent biologic function in vitro and is produced in large quantities by tissue culture cells. The ILZ portion of the chimeric protein was initially a trimerization domain obtained from a yeast sequence. In the past few years we have produce a fully human OX40 ligand protein and it was tested for in vivo biologic activity in non-human primates and had potent activity. The ultimate goal of the current research is to produce clinical grade human OX40L:Ig to test in clinical trials for patients suffering from prostate cancer. With that goal in mind we made a GMP compliant cell line that produces large quantities of the protein within the first year of funding. Future work will include testing this protein in monkey primates for toxicology studies, which are typically mandated by the FDA prior to approval for phase I studies to be conducted in cancer patients.

KEYWORDS: Cancer immunotherapy, OX40, T cell costimulation

OVERALL PROJECT SUMMARY: The third year of funding of this application was mainly spent on understanding the importance of OX40L:Ig fusion protein dosing both in terms of amount and schedule. In previous work funded by the DOD we characterized the optimal protein sequences that gave the most potent biologic activity for the OX40L:Ig fusion protein. This protein was subsequently tested in non-human primate

studies and potent biologic activity was observed, which allowed for confidence to move forward with cell line development and protein production.

During this grant's funding cycle we licensed the OX40L:Ig fusion program to an industry sponsor for testing in cancer patients (deal signed in Oct, 2011). The license deal is a collaborative project between the two groups and is especially advantageous in terms of increased support for the program as far as taking this platform through phase I, II, and III clinical trials. The sponsor's protein chemistry group has developed several antibodies and Ig fusion proteins and they have vast experience communicating with the FDA to gain approval for clinical testing. In the previous year of funding we showed that Fc-receptor binding of this fusion protein was important for its in vivo biologic activity. Based on this data our industry partner produced two protein constructs with two different Fc-tails that would interact with Fc-receptors in vivo. One of these constructs showed improved half-life and biologic activity in monkey studies and the superior OX40L:Ig construct was produced under GMP conditions for a monkey toxicity assessment. The toxicity study was completed and submitted to the FDA as part of a regulatory package to commence with a phase I study in cancer patients. In September 2014 the first cancer patient was dosed with the OX40L:Ig fusion protein. The details of this trial are documented on clinicaltrials.gov website and the link is provided, https://clinicaltrials.gov/ct2/show/record/NCT02221960?term=6383&rank=1. We are extremely excited that this protein is now being tested in cancer patients and several new trials will follow if indeed this protein is well tolerated.

In order to determine the optimal dosing and timing of this agent we performed a number of experiments to help inform our partners about this agent's activity prior to the clinical trial. As compared to an antibody the murine OX40L:Ig fusion protein has a short half-life (approximately 24hrs), therefore we first compared injecting this protein every day for 5 consecutive days versus 5 injections delivered every other day (these injections were initiated 3 days after tumor inoculation). We found that delivery of this agent on consecutive days enhanced its activity (data not shown) and hence in subsequent experiments we have injected this agent on consecutive days. We next tested whether we increased therapeutic benefit of the drug if it was delivered for 5 consecutive days vs 14 consecutive days. As shown in Figure 1 there was no difference in survival whether mice

were treated for 5 vs 14 consecutive days and the anti-murine OX40 Ab (OX86) appeared a little more therapeutic (OX86 has a 7-day half life). One anecdotal finding in this study was that the 14-day administration appeared to slow tumor growth in almost all mice compared to the Rat Ig control but eventually the mice survived at a similar percentage to mice treated for 5 consecutive days. Based on our initial study and on an impending 30-day dose monkey toxicity study by our partners we evaluated whether giving the drug for 30 consecutive days would achieve similar survival rates to the anti-murine OX40 Ab and be save. As shown in Figure 2 we found that extending the OX40L:Ig dose regimen to 30 days increased its efficacy to a similar potency observed with the OX40 Ab and no adverse side affects were observed in these mice. Finally, we increased the OX40L:Ig fusion protein dose in tumor-bearing mice to test the optimal dose to be delivered during 5 consecutive day dosing scheme (Figure 3). We found that increasing the dose to 400 ug/mouse increased the therapeutic efficacy of this agent. We have not tested doses above 400 ug/mouse but it appears increasing the dose increases the efficacy of this agent. Hence, an extensive dose escalation will be performed within the phase I clinical trial described earlier.

The dosing and timing data presented in this report was shared with our corporate partner and based on these results they developed a dose ranging phase I clinical trial that is currently being tested in cancer patients. One difference between the mouse OX40L:Ig protein and the one being tested in humans is that the human OX40L:Ig has an increased half-life, almost 3-times that of the mouse protein. Hence our partner felt based on dosing and timing that they would be able to gain beneficial therapeutic effects by administering the human protein once every two weeks for a 6-month period (there was also monkey data to support this scheme). With the phase I trial underway our future goals will test combinations with other immune enhancing agents in order to optimize the immunotherapeutic potential of OX40 agonists in tumor-bearing hosts, this goal will be accomplished in the upcoming year.

KEY RESEARCH ACCOMPLISHMENTS:

- The human OX40L:Ig fusion protein has entered into a phase I clinical trial in cancer patients.
- Prolonged administration and increasing the dose of the OX40L:Ig fusion increased its therapeutic activity in tumor-bearing mice.
- Prolonged administration of the OX40L:Ig fusion (up to 30 consecutive days) was well tolerated by mice.

CONCLUSION:

In summary, we have shown that prolonged infusion of the OX40L:Ig fusion protein is well tolerated and can increase the therapeutic efficacy of this molecule. We also found that increasing the dose of this protein also increased its therapeutic efficacy. Most importantly the first cancer patient was dosed with the human OX40L:Ig fusion protein and as the dose increases in these patients we hope to see increasing therapeutic efficacy as was observed in the mouse studies provided in this document.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS: N/A

INVENTIONS, PATENTS AND LICENSES: N/A

REPORTABLE OUTCOMES:

The experiments planned within this proposal are to develop an OX40L:Ig fusion protein that can be injected into cancer patients. Hence, most of the experiments somewhat confirm what is already known in the literature and thus are difficult to publish. The data generated in the previous reporting year regarding the OX40L:Ig fusion protein needing to bind the Fc-gamma receptor for biologic activity is a novel outcome and we were planning on submitting this finding for publication. However, prior to submitting our manuscript, another lab published a very similar finding, which reduced the novelty of this discovery.

OTHER ACHIEVEMENTS: N/A

REFERENCES: N/A

APPENDICES: N/A

Figures:

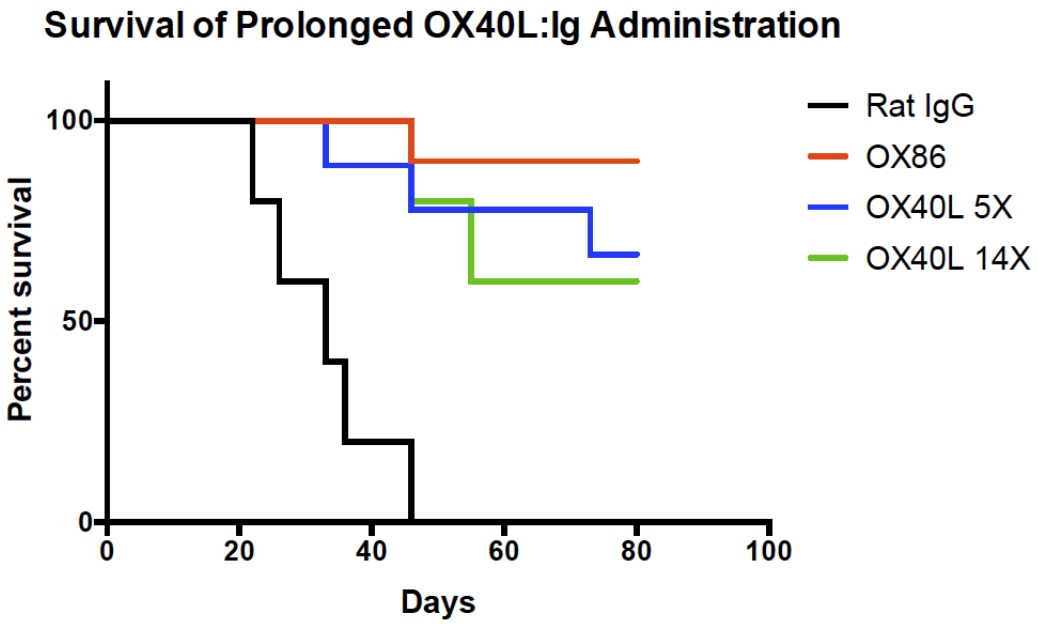

Figure 1: Increasing OX40L:Ig fusion protein from 5 to 14 consecutive days did not appreciably affect its therapeutic activity. BL/6 mice were injected with MCA205 tumors s.c. and treated with two injections of an OX40 Ab (OX86) (N= 10) or rat Ig (N = 5) 3 and 7 days later. Tumor-bearing mice were also treated with the OX40L:Ig fusion protein 3 days after tumor inoculation for 5 (N = 9) or 14 (N = 10) consecutive days. The mice were followed for tumor growth and were sacrificed when the tumor reached 150 mm2 or ulcerated.

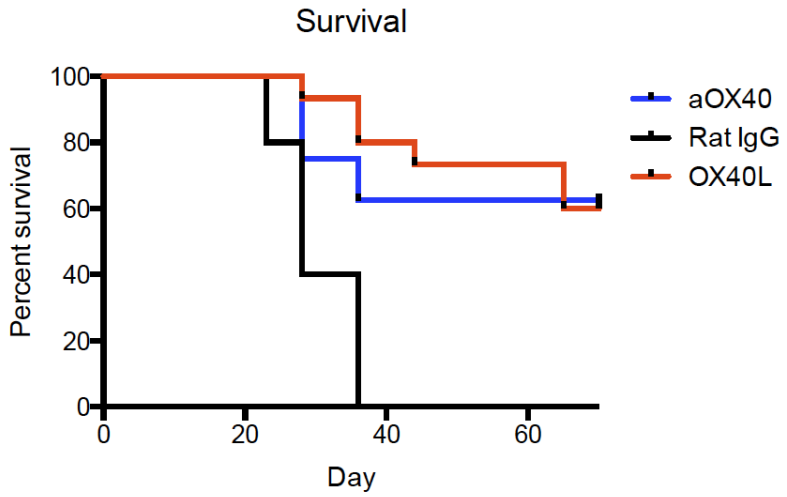

Figure 2: Increasing OX40L:Ig fusion protein to 30 consecutive days gave similar therapeutic efficacy to the anti-OX40 Ab. BL/6 mice were injected with MCA205 tumors s.c. and treated with two injections of an OX40 Ab (OX86) (N= 8) or rat Ig (N = 6) 3 and 7 days later. Tumor-bearing mice were also treated with the OX40L:Ig fusion protein 3 days after tumor inoculation 30 consecutive days N =18. The mice were followed for tumor growth and were sacrificed when the tumor reached 150 mm2 or ulcerated.

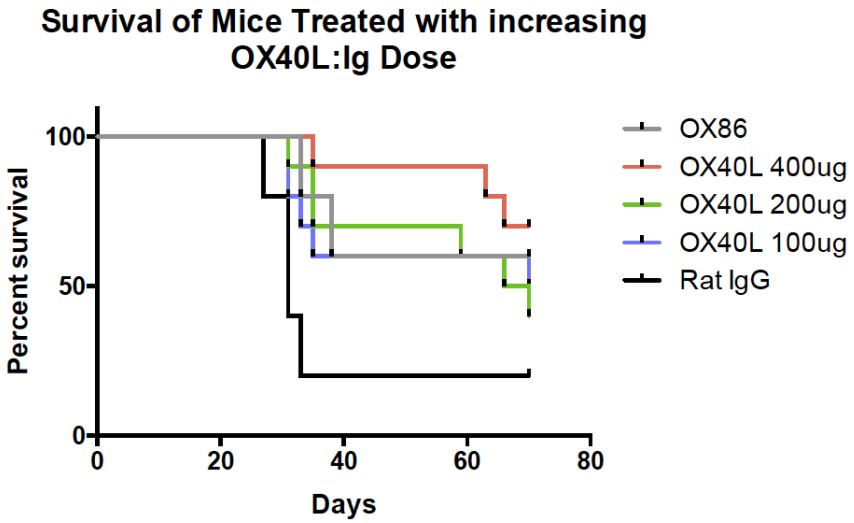

Figure 3: Increasing the dose of the OX40L:Ig fusion increases the therapeutic efficacy. BL/6 mice were injected with MCA205 tumors s.c. and treated with five daily injections of OX40L:Ig fusion protein at 100 (N = 10), 200 (N = 11), and 400 ug (N = 9) or rat Ig (N = 5) 3 days after tumor inoculation. The anti-OX40 Ab (OX86) was delivered as previously described in Figures 1 and 2. The mice were followed for tumor growth and were sacrificed when the tumor reached 150 mm2 or ulcerated.